

IN THE CLAIMS:

Please amend claims 1 and 8.

Please cancel claim 5.

Please add new claim 25.

1. (Currently amended) A method for the detection of the presence or absence of a colorectal cell proliferative disorder or metastasis, comprising determining, in a genomic DNA sample of a subject, a CpG methylation status of two genes ~~comprising~~ ALX4 and TPEF and/or their regulatory sequences, and ~~deducing, based on said determined~~ comparing the methylation status with that of a normal colorectal DNA sample, wherein a difference in the methylation status between the sample from the subject and the normal sample is indicative of the presence or absence a colorectal cell proliferative disorder or metastasis.

2. (Canceled)

3. (Canceled)

4. (Withdrawn) A method for the analysis of colorectal cell proliferative disorders, comprising determining the CpG methylation status of the gene ALX4 and/or its regulatory sequences, wherein based on said determined methylation status the presence or absence of a colorectal cell proliferative disorder or metastasis is deduced.

5. (Canceled)

6. (Withdrawn)

7. (Withdrawn)

8. (Currently amended) The method of claim 1 ~~any one of claims 1, or 4 and 5,~~ comprising:

contacting genomic DNA or a fragment thereof, obtained from a subject, with one reagent or

a plurality of reagents for distinguishing between methylated and non methylated CpG dinucleotide sequences within at least one target sequences of the genomic DNA or fragment thereof, wherein the target sequence comprises, or hybridizes under stringent conditions to, at least 16 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS:7, 8, 9, 10, 15, 16, 17 and 18, said contiguous nucleotides comprising at least one CpG dinucleotide sequence; and

determining, based at least in part on said distinguishing, the methylation state of at least one target CpG dinucleotide sequence, or an average, or a value reflecting an average methylation state of a plurality of target CpG dinucleotide sequences, whereby detecting, or detecting colon cell proliferative disorders or metastasis is afforded.

9. (Original) The method of claim 8, wherein distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises converting unmethylated cytosine bases within the target sequence to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties.

10. (Original) The method of claim 8, wherein the biological sample is selected from the group consisting of cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids, stool, blood, and combinations thereof.

11. (Previously presented) The method of claim 8, wherein distinguishing between methylated and non methylated CpG dinucleotide sequences within the at least one target sequence comprises use of at least one nucleic acid molecule or peptide nucleic acid (PNA) molecule comprising, in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:7, 8, 9, 10, 15, 16, 17 and 18, and complements thereof.

12. (Previously presented) The method of claim 8, comprising:

extracting or otherwise isolating genomic DNA from a subject biological sample having genomic DNA;

treating the isolated genomic DNA, or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated in the 5-position thereof to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties;

contacting the treated genomic DNA, or the treated fragment thereof, with an amplification enzyme and at least two primers comprising, in each case a contiguous sequence of at least 9 nucleotides that is complementary to, or hybridizes under stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:7, 8, 9, 10, 15, 16, 17 and 18, and complements thereof, wherein the treated genomic DNA or the fragment thereof is either amplified to produce at least one amplificate, or is not amplified; and

determining, based on a presence or absence of, or on a property of said amplificate, the methylation state of at least one CpG dinucleotide of SEQ ID NOS:2 and 3, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotides of SEQ ID NOS:2 and 3, wherein at least one of detecting, or detecting and distinguishing between colon cell proliferative disorders is afforded.

13. (Previously presented) The method of claim 12, wherein treating the genomic DNA, or the fragment thereof, comprises use of a reagent selected from the group consisting of bisulfite, hydrogen sulfite, and disulfite.

14. (Previously presented) The method of claim 12, wherein contacting or amplifying comprises use of at least one method selected from the group consisting of: use of a heat-resistant DNA polymerase as the amplification enzyme; use of a polymerase lacking 5'-3' exonuclease activity; use of a polymerase chain reaction (PCR); and generation of a amplificate nucleic acid molecule carrying a detectable labels.

15. (Original) The method of claim 14, wherein said nucleic acid molecule or peptide nucleic acid molecule is in each case modified at the 5'-end thereof to preclude degradation by an enzyme having 5'-3' exonuclease activity.

16. (Original) The method of claim 14, wherein said nucleic acid molecule or peptide nucleic acid molecule is in each case lacking a 3' hydroxyl group.

17. (Original) The method of claim 16, wherein the amplification enzyme is a polymerase lacking 5'-3' exonuclease activity.

18. (Previously presented) The method of claim 12, wherein determining ~~in-e~~) comprises hybridization of at least one nucleic acid molecule or peptide nucleic acid molecule in each case comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:7, 8, 9, 10, 15, 16, 17 and 18, and complements thereof.

19. (Original) The method of claim 18, further comprising extending at least one such hybridized nucleic acid molecule by at least one nucleotide base.

20. (Previously presented) The method of claim 12, wherein determining ~~in-e~~), comprises sequencing of the amplificate.

21. (Previously presented) The method of claim 12, wherein contacting or amplifying comprises use of methylation-specific primers.

22. (Withdrawn)

23. (Canceled)

24. (Previously Presented) The method of claim 8, wherein distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises the use of methylation sensitive restriction enzymes.

25. (New) A method for determining an increased likelihood of colorectal cancer in a subject comprising:

determining methylation levels of ALX4 and TPEF in a biological sample comprising colorectal cellular DNA obtained from the subject; and

comparing the methylation level of ALX4 and TPEF in the sample with the methylation level in normal colorectal cellular DNA, wherein a higher degree of methylation in the sample compared to the normal colorectal cellular DNA is indicative of an increased likelihood of colorectal cancer.